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Evaluation of CD34 expression in pediatric acute lymphoblastic leukemia: A cross-sectional study on its correlation with clinical outcomes at central teaching hospital of pediatrics, Baghdad

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Abstract
Acute lymphoblastic leukemia (ALL) is the most prevalent pediatric cancer, comprising about 25% of all cancer diagnoses in children under 15 years. This study investigates the expression of CD34, a marker associated with hematopoietic stem and progenitor cells, in pediatric ALL and its correlation with clinical outcomes at the Central Teaching Hospital of Pediatrics in Baghdad. Despite its recognized role in hematological malignancies, the relationship between CD34 expression and clinical outcomes in pediatric ALL remains inadequately explored, particularly in the Iraqi context. We conducted a cross-sectional analysis involving 60 children aged 12 years or younger, newly diagnosed with ALL and not yet undergoing chemotherapy. Flow cytometry was utilized to assess CD34 expression alongside clinical data, including symptoms, hematological parameters, and treatment responses. Our findings revealed CD34 positivity in 75% of the patients, significantly correlated with age, risk group, and immunological classification, with higher positivity rates among children under 5 years (75.6%) and B-ALL patients (89.0%). Notably, CD34 expression was associated with the presence of a mediastinal mass and CNS involvement, highlighting its potential clinical relevance. Although no significant differences were found in other hematological parameters, the study indicates that CD34 may serve as a valuable prognostic marker in pediatric ALL, particularly for risk stratification. This research underscores the need for region-specific studies to enhance understanding of prognostic factors in pediatric cancers, aiming to optimize treatment strategies and improve survival rates in the Iraqi population. Further investigation into the molecular and cytogenetic profiles of ALL patients is warranted to establish comprehensive prognostic frameworks that could facilitate personalized therapy approaches.

Keywords: CD34 expression, pediatric acute lymphoblastic leukemia, clinical outcomes, prognostic marker

Introduction
Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer, representing approximately 25% of all cancer diagnoses in children and adolescents under 15 years of age [1]. CD34, a surface glycoprotein, serves as a marker of hematopoietic stem and progenitor cells, and its expression has been frequently observed in acute leukemia [2]. The significance of CD34 expression in pediatric ALL has garnered increasing attention due to its potential role in influencing the disease's biology, prognosis, and treatment response [3]. Understanding CD34 expression in ALL is crucial for stratifying patients according to risk and personalizing therapy [4]. While previous studies have highlighted the association between CD34 positivity and poor prognosis in some hematological malignancies, its specific correlation with clinical outcomes in pediatric ALL remains a subject of debate [5].
Despite the recognized role of CD34 as a hematopoietic marker, there is insufficient data on the correlation between CD34 expression and clinical outcomes in pediatric ALL within the Iraqi population [6]. Few studies have explored this biomarker's role in predicting disease progression, treatment response, or survival rates in pediatric patients [7]. This gap in the literature, particularly within the context of developing countries, underscores the need for

region-specific research to enhance the understanding of CD34 expression's prognostic significance in pediatric ALL patients in Baghdad [8]. This study aims to address this gap by evaluating CD34 expression in pediatric ALL cases and determining its correlation with clinical outcomes at the Central Teaching Hospital of Pediatrics in Baghdad.

The clinical management of pediatric ALL has improved significantly over the past few decades due to advancements in chemotherapy protocols, risk stratification, and supportive care [9]. However, relapse remains a challenge, particularly in high-risk patients [10]. Identifying biomarkers like CD34 that can predict clinical outcomes early in the disease course could lead to improved risk-adapted therapies [11]. This research is particularly relevant for the Iraqi healthcare setting, where resources for genetic and molecular profiling are limited [12]. Establishing a correlation between CD34 expression and clinical outcomes could guide more effective treatment strategies, ultimately improving survival rates and reducing relapse [13].

Several studies have examined the role of CD34 expression in acute leukemia. Research has shown that CD34-positive ALL cases may exhibit more aggressive disease phenotypes and poorer clinical outcomes [14]. A meta-analysis conducted by Prebet *et al*. found that CD34 expression is associated with a higher likelihood of minimal residual disease (MRD) positivity, which is a predictor of relapse in pediatric ALL [15]. Conversely, some studies argue that the impact of CD34 on prognosis is less significant in pediatric populations compared to adult populations [16]. However, much of this research is based on Western populations, with limited representation from the Middle East and developing countries, necessitating further investigation [17].

This study will evaluate the expression of CD34 in pediatric ALL patients at the Central Teaching Hospital of Pediatrics in Baghdad and its correlation with clinical outcomes, including remission rates, overall survival (OS), and event-free survival (EFS). The study is retrospective in design and focuses on a cohort of pediatric patients diagnosed with ALL between 2019 and 2023 [18]. A potential limitation is the reliance on archival clinical data and immunohistochemical analyses, which may affect the completeness of data [19]. Additionally, the lack of molecular and cytogenetic profiling in some cases could limit the ability to assess the full impact of CD34 expression on clinical outcomes [20].

Objectives

1. To determine the prevalence of CD34 expression in pediatric ALL cases at the Central Teaching Hospital of Pediatrics.
2. To assess the correlation between CD34 expression and key clinical outcomes.
3. To evaluate whether CD34 expression can serve as an independent prognostic factor for pediatric ALL patients.

Patients and Methods

A cross-sectional study was conducted at the Central Teaching Hospital of Pediatrics in Baghdad between January and May 2014. The study included 50 children aged 12 years or younger who were newly diagnosed with ALL and had not yet started chemotherapy. Diagnosis was confirmed based on the morphology and cytochemistry of peripheral blood (PB) and bone marrow aspirates (BMA), evaluated by consultant hematologists. Children with specific conditions, including those with ALL L3 or those who had started chemotherapy in other hospitals, were excluded.

Data collection involved a questionnaire that gathered clinical symptoms, physical signs (such as hepatosplenomegaly, lymphadenopathy, CNS involvement, and mediastinal mass), and hematological parameters like hemoglobin (HB), packed cell volume (PCV), white blood cell (WBC) count, platelet count, and serum lactate dehydrogenase (S.LDH). This information was obtained from patient files, with blood tests performed using automated analyzers.

For all participants, blood and bone marrow samples were collected upon arrival at the hematology ward. A 2.5 ml blood sample and 0.5 ml of BMA were placed in ethylene diamine tetra-acetic acid (EDTA) tubes. Peripheral blood and bone marrow films were stained and reviewed using specialized stains, and patients were classified according to the French-American-British (FAB) classification system based on leukemia morphology.

Flow cytometry was employed to analyze cell markers in the blood and bone marrow. A 0.3 ml sample of EDTA anti-coagulated bone marrow or blood was used for cytometric analysis, which identified cytoplasmic and surface markers including CD3, CD19, and CD34. This analysis was essential for distinguishing between B-cell and T-cell ALL subtypes. The samples were either processed on the same day or stored for a short period before testing. The flow cytometry was conducted using the Cyflow® Cube 6 system, a compact device capable of high-precision cellular analysis with multiple optical parameters and fluorescence channels. The procedure involved staining cells with antibodies, followed by incubation, fixation, and lysing of the samples before the flow cytometer analyzed the data.

The gating strategy in flow cytometry isolated leukemic blasts by plotting forward versus side scatter, and further differentiated B-ALL (CD19 positive) from T-ALL (CD3 positive) using appropriate markers. A cut-off of 20% was set for surface markers CD34 and CD19, while 10% was used for the cytoplasmic marker CD3.

Data analysis was performed using SPSS version 25. Descriptive statistics were applied to summarize the participant characteristics, and chi-square and t-tests were used to assess relationships between variables. A p-value below 0.05 was considered statistically significant. The study adhered to ethical guidelines, with approval obtained from the hospital administration and the Ministry of Health of Iraq. Patient confidentiality was maintained, and data were used solely for research purposes.

Results

In the studied sample of 60 individuals, CD34 marker expression was positive in 75.0% (45) and negative in 25.0% (15). A significant association was found between CD34 expression and age group, with children under 5 years showing a higher positive rate (75.6%) compared to those aged 5-10 years (13.3%) and over 10 years (11.1%) ($p = 0.029$). CD34 expression was also significantly associated with risk group, with 64.4% of standard-risk patients being positive compared to 35.6% in the high-risk group ($p = 0.012$). Immunological classification revealed a strong correlation, with 89.0% of B-ALL patients showing CD34 positivity versus 11.0% in T-ALL ($p = 0.001$). Other factors such as gender, FAB classification, and response to therapy did not show significant differences. (Table 1)

Table 1: Association of CD34 marker expression with demographic, clinical, and immunological characteristics in pediatric patients

Variable		CD34 Marker		Total 60 (100.0)	P- Value*
		Positive 45 (75.0)	Negative 15 (0.25)		
Age Group (Years)	< 5	34 (75.6)	5 (33.3)	39 (65.0)	0.029
	5-10	6 (13.3)	5 (33.3)	11 (19.0)	
	> 10	5 (11.1)	5 (33.3)	10 (16.0)	
Gender	Male	29 (64.4)	7 (46.3)	36 (60.0)	0.223
	Female	16 (35.6)	8 (53.8)	24 (40.0)	
FAB Classification	L1	8 (17.8)	4 (26.7)	12 (20.0)	0.456
	L2	37 (82.2)	11 (73.3)	48 (80.0)	
Risk Group	Standard	29 (64.4)	6 (40.0)	35 (58.0)	0.012
	High	16 (35.6)	9 (60.0)	25 (42.0)	
Immunological Classification	B-ALL	40 (89.0)	8 (53.3)	48 (80.0)	0.001
	T-ALL	5 (11.0)	7 (46.7)	12 (20.0)	
Response to Therapy	Yes	42 (93.3)	12 (80.0)	54 (90.0)	0.136
	No	3 (6.7)	3 (20.0)	6 (10.0)	

*Significant difference between percentages using Pearson Chi-square test at 0.05 level

In pediatric patients, CD34 marker expression was positive in 45 patients (75%) and negative in 15 patients (25%). While pallor, bruising, hepatosplenomegaly, fever, and lymphadenopathy did not show significant associations with CD34 expression (p-values > 0.05), the presence of a mediastinal mass (p = 0.036) and CNS involvement (p = 0.005) were significantly associated with CD34 positivity. Specifically, 26.6% of patients with mediastinal masses and 40% of those with CNS involvement were CD34 positive, indicating potential clinical relevance for these parameters. (Table 2)

Table 2: Association of CD34 marker expression with clinical parameters in pediatric patients

Clinical Parameter		CD34 Marker		P-Value*
		Positive 45 (75.0)	Negative 15 (25.0)	
Pallor	Present	24 (53.3)	11 (73.3)	0.173
	Absent	21 (46.7)	4 (26.7)	
Bruising	Present	8 (17.7)	6 (40.0)	0.078
	Absent	37 (82.3)	9 (60.0)	
Hepatosplenomegaly	Present	21 (46.7)	9 (60.0)	0.371
	Absent	24 (53.3)	6 (40.0)	
Fever	Present	23 (51.1)	11 (73.3)	0.132
	Absent	22 (48.9)	4 (26.7)	
Lymphadenopathy	Present	25 (55.6)	10 (66.7)	0.449
	Absent	20 (44.4)	5 (33.3)	
Mediastinal mass	Present	3 (6.7)	4 (26.6)	0.036
	Absent	42 (93.3)	11 (73.4)	
CNS Involvement	Present	2 (4.4)	6 (40.0)	0.005
	Absent	43 (95,6)	9 (60.0)	

*Significant difference between percentages using Pearson Chi-square test at 0.05 level

In this study, CD34 marker expression showed a significant association with certain hematological parameters. The mean white blood cell (WBC) count was significantly lower in CD34-positive patients (42.2±5.9 × 10^9/L) compared to CD34-negative patients (127.3±17 × 10^9/L), with a P-value of 0.001. Hematocrit (HCT), hemoglobin, and platelet counts did not show significant differences between the groups, with P-values of 0.284, 0.287, and 0.753, respectively. Blast cell percentages were slightly higher in CD34-negative patients (68.7±6.5%) than in CD34-positive ones (63.2±11.5%), but this difference was not statistically significant (P = 0.110). Serum lactate dehydrogenase (LDH) levels ≥ 400 U/L were more frequent in CD34-negative patients (40%) compared to CD34-positive patients (24.4%), but this difference was also not statistically significant (P = 0.246). (Table 3)

Table 3: Association of CD34 marker expression with hematological parameters in pediatric patients

Laboratory Parameters	CD34 Marker		P-value
	Positive (n=45) Mean±SD	Negative (n=15) Mean±SD	
WBC count (× 10^9/L)	42.2±5.9	127.3±17	0.001
HCT (%)	27.5±3.1	26.4±3.3	0.284
Hemoglobin(g/dl)	9.1±3.8	6.9±2.2	0.287
Platelets (x 10 ^{9}/L)	53.5 ±9.3	52.2.0±11.3	0.753
Blast cells (%)	63.2±11.5	68.7±6.5	0.110
* Significant difference between two independent means using Students-t-test at 0.05 level.			
Serum Lactate Dehydrogenase Level			
< 400 (U/L)	34 (75.6)	9 (60.0)	0.246
≥ 400 (U/L)	11 (24.4)	6 (40.0)	

Discussion

This study found that CD34 marker expression was positive in 75% of pediatric patients, predominantly in those under 5 years (75.6%) and among B-cell acute lymphoblastic leukemia (B-ALL) patients (89%). A significant association was observed between CD34 positivity and key clinical features such as the presence of a mediastinal mass (P = 0.036) and central nervous system (CNS) involvement (P = 0.005). Additionally, CD34-positive patients had significantly lower white blood cell (WBC) counts compared to CD34-negative patients (P = 0.001). However, no significant differences were noted in hematocrit, hemoglobin, or platelet levels between the two groups.

The strong association between CD34 positivity and younger age, especially in patients under 5 years, is consistent with existing literature, which suggests that CD34 expression is more prevalent in pediatric patients with B-ALL compared to older children and adults [21]. Moreover, the correlation between CD34 expression and B-ALL is in line with previous findings that link CD34 positivity to this immunophenotype, which has been associated with better treatment response and prognosis [22]. However, the lack of significant differences in other hematological parameters, such as hemoglobin and platelet levels, contrasts with studies that found more pronounced differences [23].

The lower WBC count in CD34-positive patients suggests that these patients might have a less aggressive disease course, as higher WBC counts are typically associated with a worse prognosis in leukemia [24]. The association of CD34 with younger age groups may be related to the higher proliferative capacity of hematopoietic stem cells in early childhood, where CD34 serves as a marker of stem cell immaturity [24]. The significant correlation with CNS involvement and mediastinal masses implies that CD34 expression may play a role in the dissemination of leukemia, although further studies are required to clarify this mechanism [25].

The findings suggest that CD34 marker expression can be used as a potential prognostic indicator in pediatric leukemia, particularly for identifying patients at higher risk of CNS involvement and mediastinal masses [26]. This could help guide clinical decision-making regarding more aggressive treatment or CNS-directed therapy [27]. The association between CD34 positivity and B-ALL also reinforces the potential of CD34 as a target for future immunotherapies [28].

This study's strengths include a robust sample size for a pediatric population (n=60), a well-defined stratification of patients by risk group and immunophenotype, and the use of established diagnostic markers like CD34, which enhances the study's clinical relevance. Additionally, the study's focus on the correlation between CD34 expression and specific clinical features such as CNS involvement adds depth to the existing literature. Limitations include the relatively small sample size for CD34-negative patients (n=15), which may have limited the statistical power for detecting differences in certain parameters. Moreover, the study did not account for potential confounders such as prior treatments or genetic mutations, which could influence CD34 expression and the associated outcomes. The retrospective nature of the study may also introduce bias in the collection of clinical data. Further research should focus on larger, multi-center studies to confirm the associations between CD34 expression and clinical outcomes, particularly in different subtypes of pediatric leukemia. Prospective studies could explore the role of CD34 in disease progression and treatment response, potentially integrating genetic profiling to identify underlying mechanisms. Additionally, studies could investigate the role of CD34-targeted therapies in pediatric leukemia patients.

Conclusions

The study investigated CD34 marker expression in 60 pediatric patients, revealing that 75% exhibited positive expression. Notably, positivity was significantly associated with younger age groups, particularly in children under 5 years (75.6%), and with B-cell acute lymphoblastic leukemia (B-ALL), where 89% showed positivity. Additionally, CD34 expression correlated with clinical features such as mediastinal masses ($p = 0.036$) and central nervous system (CNS) involvement ($p = 0.005$). CD34-positive patients had significantly lower white blood cell (WBC) counts compared to CD34-negative patients ($42.2\pm5.9 \times 10^9$/L vs. $127.3\pm17 \times 10^9$/L, $p = 0.001$), while no significant differences were observed in hematocrit, hemoglobin, or platelet levels. These findings highlight CD34 expression as a potential prognostic marker in pediatric leukemia, reinforcing its relevance in understanding disease biology and progression. The strong link between CD34 positivity, younger age, and B-ALL suggests its utility in clinical assessments and treatment strategies. Furthermore, the significant association with mediastinal masses and CNS involvement indicates that CD34 could serve as an indicator of disease severity and potential complications. Given these associations, CD34 expression may aid in identifying patients at higher risk for severe outcomes, guiding therapeutic interventions. Overall, the study underscores the clinical importance of CD34 expression in pediatric leukemia and calls for further research to elucidate the underlying mechanisms influencing CD34 expression in this context.

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